

THE DETERMINATION OF NITROBENZOL IN PEANUT OIL¹

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In the regular routine work of this laboratory, it became necessary to determine the amount of nitrobenzol present in certain varieties of peanut oil on the market. Because of its cheapness, a very large amount of this oil is consumed by the people of Porto Rico, especially the poorer classes, as a substitute for olive and other higher-priced oils. Inspection showed that a considerable percentage of the oil on the market was adulterated by the addition of nitrobenzol, which has been added for the purpose of giving a pleasant odor, and of deceiving the purchaser into thinking that the product was oil of bitter almonds. An examination of these products led to the working out of the following method.

Nitrobenzol is easily volatile with steam, and can be distilled from an oil by this means. However, experiments showed that there is considerable loss from this procedure: (1) because, it is very difficult to remove the last portions, and (2) because there is a continual, although slight loss by volatilization as is evidenced by the pronounced odor of the distillate. Since the distillation must be carried on for some time this continual volatilization causes an appreciable loss.

Nitrobenzol is easily reduced to aniline in hydrochloric acid solution by such reducing agents as stannous chloride, and metals. Attempts were made to recover this aniline by distillation with steam, but the same difficulties were encountered, as mentioned above. However, by the use of suitable immiscible solvents it was found that the aniline could be separated from the oil, evaporated to dryness from a hydrochloric acid solution, and weighed as aniline hydrochloride. Although the method does not give a complete recovery of nitrobenzol, it is so nearly so that for practical purposes it may be considered as quantitative.

To peanut oil, free from nitrobenzol and aniline, were added varying amounts of nitrobenzol, giving mixtures containing from 0.125 per cent to 1.000 per cent of the latter. These were then analyzed according to the following method: Thirty grams of oil containing nitrobenzol are weighed into a counterpoised, glass-stoppered Erlenmeyer flask of 150 cc. capacity. Five grams of zinc dust are added, and the oil gently agitated in order to distribute the finely divided metal throughout the mixture. Ten cc. of concentrated hydrochloric acid are then poured in, and the whole mixed quickly and thoroughly by three or four rapid whirls. Finally, 10 cc. of water are added, and the entire contents shaken vigorously, care being taken to avoid loss, since the heat development may cause the stopper to loosen.

Should the odor of nitrobenzol disappear within a few seconds, the amount of same present is very small, and but little more shaking is required for complete reduction. If, however, it persists for over three minutes, the amount is so large that continual shaking for fifteen minutes is necessitated. In either

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event, at the end of this time the liquid contents of the flask are transferred to a separatory funnel by means of 50 cc. of ether and 25 cc. of water, care being taken to leave behind in the flask as much of the undissolved zinc as possible, which is washed into a second separatory funnel with 25 cc. of ether and 25 cc. of 5 per cent hydrochloric acid solution. The aqueous layer from the first separatory funnel is filtered through a wet filter into a third, and that from the second into the first. The contents of the second are then washed with 25 cc. and 10 cc. of 5 per cent hydrochloric acid, in two successive portions, which are, in turn, used for washing the oil-ether mixture in the first. The separate extractions and washings should be performed by shaking for two minutes, cautiously, as an emulsion is very liable to form if the shaking is done vigorously. The filtrate is a perfectly clear solution of zinc chloride and aniline hydrochloride in dilute hydrochloric acid, with traces of oil which passed through the filter.

This is now allowed to run slowly into 50 cc. of strong sodium hydroxide solution (100 grams of sodium hydrate to 150 cc. of water) which must be kept cool and shaken constantly. It was found very convenient to have the alkali in another separatory funnel. The resulting solution should be clear, or nearly so. The aniline separates as an oily layer, if the amount present is considerable, and the zinc remains dissolved as sodium zincate. Extraction with ether is made, using 60, 40, 30 and 20 cc., and pouring off from the top into a perfectly clean and dry separatory funnel. If, after standing for half an hour, small drops of water should be visible on the inner wall, the ether must be transferred to another, thus securing a solution of aniline free from inorganic salts. Final extraction is now made, using 4, 3, 2 and 1 cc. of 10 per cent hydrochloric acid solution successively, and shaking each time for 2 minutes. The acid extractions are evaporated on the water-bath in a tared platinum dish, which is kept covered until all the ether has boiled away and danger from loss by spattering has ceased.

No.	SAMPLE TAKEN				PEANUT OIL RECOVERED				Weight non-volatile residue, Mg.
	Grams oil	Per cent C ₆ H ₅ NO ₂	Gram C ₆ H ₅ NO ₂	Gram C ₆ H ₅ NH ₂ HCl	Gram C ₆ H ₅ NO ₂	Per cent C ₆ H ₅ NO ₂	Per cent recovery		
1	30	1.000	0.3000	0.3030	0.2879	0.960	96.0	0.2	
2	30	0.500	0.1500	0.1521	0.1445	0.482	96.3	0.0	
3	30	0.250	0.0750	0.0728	0.0692	0.230	92.2	0.7	
4	30	0.250	0.0750	0.0729	0.0693	0.231	92.4	0.0	
5	30	0.125	0.0375	0.0371	0.0352	0.117	94.0	0.6	
LARD OIL									
6	30	1.000	0.3000	0.2960	0.2812	0.937	93.7		
7	30	0.500	0.1500	0.1486	0.1412	0.471	94.1		

The evaporation must be watched very carefully at the finish, the dish being removed just before dryness is reached, at a point where the whole mass will just crystallize when allowed to cool. If carried further, there is danger of loss by volatilization. The residue is dried to constant weight in a soda lime desiccator: this requires from 16 to 35 hours, depending upon the amount of residue. On ignition at a low red heat,

the amount of inorganic material is obtained, which, when subtracted from the total residue, gives the weight of aniline hydrochloride.

The accompanying table gives the results of analyses of mixtures of peanut oil and nitrobenzol of known strength, and also two of lard oil and nitrobenzol.

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THE DETERIORATION OF SODA WATER DUE TO MICRO-ORGANISMS

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During the last two years many letters have been received at this laboratory from soda water bottlers asking for information relative to the cause and prevention of the so-called "diseases" of soda water. In a few instances, at least, bottling works have been forced to close down their business, owing to the fact that the product put out by them continually "went bad." As it is of economic importance to the various soda water manufacturers throughout the country and indirectly of interest to the consumer, an invitation was extended to various bottling works in the state to send in to the laboratory samples of spoiled soda water whenever found. Accordingly, a large number of samples were received and examined. It was found that the spoiled beverages may be classified into three groups, namely: *First*, stringy pop; *Second*, pop with sediment and turbidity; and *Third*, pop in which sufficient fermentation had developed to blow off the caps or break the bottles. The authors have been unable to find records of any previous work on this subject.

A microscopical examination of several samples revealed the fact that stringy pop, in these particular cases, was caused by the presence of a one-celled algae, "Tetraspora." Data on file in the water laboratories showed that the water supply used in the manufacture of the soda water was infested with an excessive growth of this algae. The stringiness was due to the mucilaginous nature of the cell walls which hold the cells together in large colonies. It can readily be seen that any algae-infested water, used directly, will give the manufacturer trouble. The use of distilled water is the best and easiest solution of this difficulty. Filters will not protect the bottler from this trouble entirely as the algae grow through the filters.

Those samples of soda water, showing only sediment and turbidity or in many cases sediment alone, were shaken, carefully opened and portions of the contents plated out on agar and other portions were inoculated into saccharose and dextrose broth fermentation tubes. Microscopical examinations were also made of the sediment settling to the bottom of the bottle. The agar plates showed the presence of 30,000 to 45,000 yeasts per cubic centimeter while the microscopical examination of the sediment established the fact that it was made up, in every case, of yeasts. Several strains of bacteria were isolated along with the yeasts and kept for further study as will be described presently.

While many bottlers reported losses due to blowing off of caps to bottles no samples thus affected were obtained, but instead a successful attempt was made to

produce this effect by inoculating various kinds of soda water under normal conditions, with three laboratory strains of saccharose fermenting colon organisms, which had been isolated from polluted water and sewage. The yeasts and bacteria isolated from samples showing sediment and in some cases marked turbidity were separately inoculated each into a respective set of samples to check up the cultural and microscopical findings mentioned above. A set of controls was also carried. Two varieties of soda water were used in these experiments, Cocoa Cola and Cream. These were inoculated in sets of four bottles each with the following organisms:

Set Lab. No.

1	101	Saccharose fermenting colon organism.
2	102	Saccharose fermenting colon organism.
3	107	Saccharose fermenting colon organism.
4	5428	Yeast (from ginger ale).
5	5429	Saccharose fermenting bacillus. Acid. No gas (from cream pop).
6	5432	Yeast (from lemon soda).
7	5426	Saccharose fermenting bacillus. Acid. No gas (from ginger ale).
8	5427	Saccharose fermenting bacillus. Acid. No gas (from ginger ale).
9		Controls.

NOTE: These experimental samples were bottled under normal factory conditions, with the exception that all bottles were sterilized.

When soda "goes bad" it does so almost invariably in hot weather, hence these bottles were placed in an incubator and kept at 37° C. for a week and then placed at room temperature and kept for several months with the following results:

SET	ACTION
1	Developed slight turbidity only.
2	Developed slight turbidity only.
3	Developed slight turbidity and sufficient gas produced to lift caps.
4	Heavy sediment produced, made up of yeast and cap from one bottle lifted.
5	Turbidity only.
6	Sediment yeast.
7	Turbidity only.
8	Contaminated with yeasts, probably from bottling machine.
9	Normal pop. No sediment or turbidity.

NOTE: Active-gas producing colon organisms and yeast were found.

It would seem from the above that while not all strains of saccharose fermenting colon organisms will produce sufficient gas to lift the caps on the bottles, yet an extremely good gas-producing strain may bring this about and also that some strains of yeast will produce this result. Hence the use of polluted water might, in addition to endangering the health of the consumer, render the produce unsalable owing to presence of large numbers of colon organisms.¹ In a previous paper by the authors, it was noted that when a certain strain of *B. coli* was inoculated into pop it rapidly died out; this, however, was a non-saccharose fermenting organism and did not produce turbidity in the pop.

It is also evident from these experiments that sediment may be produced in pop owing to presence of certain wild yeasts, and also that any bacteria which ferment saccharose with the production of acid but no gas may render the pop turbid and unsalable.

Inasmuch as it is an established fact that algae grows only in the light it is important that all distilled

¹ THIS JOURNAL, 3, 495.